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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING ESTETROL DERIVATIVES FOR USE IN CANCER THERAPY

(57) Abstract: The present invention relates to a method of treating or preventing estrogen-sensitive tumours in a mammal, said method comprising the administration of a therapeutically effective amount of an estrogenic component to said mammal, wherein the estrogenic component is selected from the group consisting of: substances represented by the following formula (I) in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms; precursors capable of liberating a substance according to the aforementioned formula when used in the present method; and mixtures of one or more of the aforementioned substances and/or precursors. The estrogenic component according to the invention does not have undesirable proliferative effects on breast and/or endometrial tissue and displays sufficient estrogenicity to prevent that its administration will

PHARMACEUTICAL COMPOSITION COMPRISING ESTETROL DERIVATIVES FOR USE IN CANCER THERAPY

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method for treating or preventing estrogen-sensitive tumours in a mammal by administering an effective amount of a special estrogenic component to said mammal. The method is particularly suited for treating or preventing breast cancer and endometrial cancer.

BACKGROUND OF THE INVENTION

Breast cancer is one of the leading causes of cancer mortality among Western women, and is predicted to become a leading cause of cancer death in Oriental women in countries such as Japan in the near future. The American Cancer Society estimates that 1 in 9 women face a lifetime risk of this disease, which will prove fatal for about one-quarter of those afflicted with the disease. Breast tumours as well as some other tumours (including uterine cancer, ovarian cancer, endometriosis, uterine fibroids, benign prostatic hyperplasia and melanoma), are known to be estrogen-sensitive, meaning that the formation and growth of such tumours is stimulated by estrogens such as 17β-estradiol. 17β-estradiol is an estrogen that is endogenous to the human body and that is found in both females and males.

Estrogens are known to increase the risk of e.g. breast and endometrial tumours by inducing an estrogen receptor mediated increase in the frequency of breast and endometrial cell division (proliferation). Cell division is essential in the complex process of genesis of human cancer since it *per se* increases the risk of genetic error, particularly genetic errors such as inactivation of tumour suppressor genes.

An important element of the treatment of estrogen-sensitive tumours, is the suppression or, if possible, elimination of certain estrogen-induced effects. For this purpose, it is desirable to block receptor sites stimulated by estrogens and/or to reduce the amount of estrogen available to act at these sites.

A commonly used therapy to block receptor sites involves the administration of anti-estrogen. Anti-estrogens are a class of chemicals which inhibit estrogens from eliciting their full response in target tissues. An anti-estrogenic compound currently being utilised in the chemotherapy of estrogen-sensitive cancers is tamoxifen. Tamoxifen is a so called selective estrogen receptor modulator (SERM), meaning that the substance exhibits both estrogen antagonist and agonist properties. Although such mixed agonist/antagonists have beneficial effects in the treatment of these cancers, the estrogenic side-effects are also known to have stimulatory effects on certain cancer cell populations in the uterus and therefore, are counterproductive in some cases. SERMs that seem not to display such uterine agonistic effects are also known in the art (e.g. raloxifene), but suffer from the drawback that they can induce climacteric complaints such as hot flushes and sweats. Furthermore, such SERMs have been associated with an enhanced risk of venous thromboembolism, which is another agonistic estrogenic effect.

Reduction of estrogen concentrations in blood serum may be achieved surgically (ovariectomy, adrenalectomy, hypophysectomy) or pharmaceutically through administering high doses of progestogen, GnRH analogue or steroid pathway inhibitors. However, long term suppression of endogenous estrogen production will lead to hypoestrogenism. Furthermore, it is noted that even in the total absence of sex steroids, some receptors may be activated. See Simard and Labrie, "Keoxifene shows pure antiestrogenic activity in pituitary gonadotrophs", Mol. Cell. Endocrinol. 39: 141-144, (1985), especially page 144.

US 4,937,238 (Lemon) relates to a method of preventing breast cancer in female mammals comprising the steps of administering a compound selected from the group of drugs including (1) 4-OH estradiol; (2) d-equilenin; and (3) 17α-ethinyl estriol. A general formula is provided to describe a set of compounds (1) including 4-OH estradiol. Said formula encompasses a huge variety of estrogen-like substances, including substances that may contain 4 or more hydroxyl groups. With the exception of 4-OH estradiol no other representative of this large group of substances are discussed.

US 5,340,584 (Spicer et al.) describes a method for preventing conception or for treating benign gynaecological disorders comprising administering a GnRH composition for a first period of time in an amount effective to suppress ovarian estrogen and progesterone production, simultaneously administering an estrogenic composition in an amount effective to prevent symptoms of estrogen deficiency and simultaneously administering a progestogen in an amount effective to maintain serum level of said progestogen at a level effective to decrease endometrial cell proliferation. The US patent is primarily concerned with slow

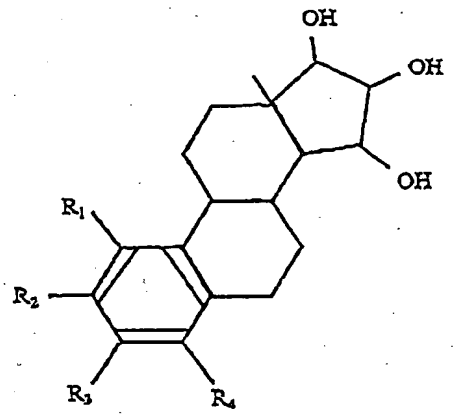
release formulations that are effective over an extended period of time of at least about two months. In a long list of estrogens that can be used in the claimed invention estetrol is mentioned.

WO 02/30355 (Kragie) describes a method of alleviating adverse side effects and/or enhancing the beneficial efficacy of an aromatase inhibitor in a subject, wherein said method comprises administering a combination of one or more aromatase inhibitors with one or more estrogen function replacement agents (EFR). A wide array of EFR agents are recited in the application, including estrogens. In a list of estrogens also estetrol is mentioned. The claimed method is said to be beneficial for treating subjects suffering from side effects and reduced therapeutic benefit of compositions comprising an aromatase inhibitor administered as a therapeutic for a large variety of disease states or clinical indications. In relation to breast cancer, which is mentioned as an example of a disease state, it is observed that aromatase inhibitors are used to diminish the production of estrogens at the site of cancerous breast tissue. Selective EFR agents such as raloxifene and estradiol metabolites are said to be beneficial as an EFR agent in tumor therapy. As regards estradiol metabolites, reference is made to an article by Lippert TH, et al. Steroids 2000; 65:357-69. Said article reports the results of a study into the effects of A-ring and D-ring metabolites of estradiol, including estetrol, on the proliferation of vascular endothelial cells. The results show that some A-ring metabolites are capable of inhibiting proliferation of cultured endothelial cells of human umbilical cord veins. No significant effect was observed for estetrol.

Estrogen antagonists will usually produce better therapeutic results than therapy which only inhibits estrogen production, e.g. GnRH analogues, aromatase inhibitors and/or progestogens. Consequently, there is a need for a drug that exhibits a more favourable combination of agonistic and antagonistic (or non-agonistic) properties than the anti-estrogens and/or SERMs that are currently available. In particular, there is a need for a drug which does not have undesirable proliferative effects on breast and/or endometrial tissue and which, at the same time, displays sufficient estrogenicity to prevent that its administration will lead to hypoestrogenism and/or climacteric complaints.

SUMMARY OF THE INVENTION

The inventors have unexpectedly found that these requirements are met by estrogenic substances that are represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms.

A known representative of this group of estrogenic substances is 1,3,5 (10)-estratrien-3, 15α,16α,17β-tetrol, also known by the names of estetrol, oestetrol and 15α-hydroxyestriol. Estetrol is an estrogen that is produced by the fetal liver during human pregnancy. Unconjugated estetrol levels in maternal plasma peak at about 1.2 ng/ml at term pregnancy and are about 12 times higher in fetal than in maternal plasma (Tulchinsky et al., 1975. J. Clin. Endocrinol. Metab., 40, 560-567).

It is very surprising that the present estrogenic substances can advantageously be used in the treatment of estrogen-sensitive tumours as the skilled person would expect estrogenic substances to enhance the formation and growth of such tumours. Since the present estrogenic substances do not appear to exhibit estrogen antagonistic properties, this finding is truly unexpected.

Although the inventors do not wish to be bound by theory, it is believed that the favourable effect of the present estrogenic component (EC) is caused by a primary mechanism by which said component competes with other estrogens for binding to cytoplasmic estrogen receptors ("ER"). The resulting ER-EC complex is believed to inhibit many of the activities of endogenous estrogen within tumour cells. Endogenous estrogens, such as 17β-estradiol, bind

with ERs to promote cellular activities such as estrogen/ER-mediated gene transcription, DNA synthesis, cancer cell growth, and increases in autocrine polypeptides such as transforming growth factor-alpha, epidermal growth factor, insulin-like growth factor-II, and other growth factors that may be involved in cell proliferation. Competitive inhibition of binding of endogenous estrogen to ERs by the present estrogenic component reduces or prevents such cancer growth inducing cellular activities by the endogenous estrogens. Due to the lack of a proliferative impact on e.g. breast tissue, the present estrogenic component prevents the transition of breast cancer cells from the early G1 phase to the mid-G1 phase of the cell cycle and exhibits a cytostatic effect on breast cancer cells.

The present estrogenic substances were found to exhibit a relatively high affinity for the ERα receptor, or conversely a relatively low affinity for the ERβ receptor. It is believed that this receptor specificity is somehow associated with the high efficacy of the present substances in the treatment of estrogen-sensitive tumours. However, the mechanisms that govern the ER signalling pathways that are responsible for this efficacy are as yet poorly understood, despite the considerable scientific effort that is ongoing in this area.

It is known that most estrogens bind to both ERs which, in the presence of tissue-specific co-activators and/or co-repressors, bind to an estrogen response element in the regulatory region of genes or to other transcription factors. Given the complexity of ER signalling, along with the tissue-specific expression of ERα and ERβ and its co-factors, it is now recognised that ER ligands can act as estrogen agonists or even as estrogen antagonists in a tissue-specific manner.

It is also now known that estrogen modulates cellular pharmacology through gene expression, and that the estrogen effect is mediated by the estrogen receptors. The effect of the estrogen receptor on gene regulation can be mediated by a direct binding of ER to the estrogen response element, binding of ER to other transcription factors such as NF-κB, C/EBPβ and through non-genomic effects involving ion channel receptors. Progress over the last few years has shown that ER associates with co-activators (e.g., SRC-1, CBP and SRA) and co-repressors (e.g., SMRT and N-CoR), which also modulate the transcriptional activity of ER in a tissue-specific and ligand-specific manner. In addition, evidence now suggests that the majority of estrogen-regulated genes do not have a classical estrogen response element. In such cases, ER interacts with the transcription factors critical for regulation of these genes. Transcription factors known to be modulated in their activity by ER include, for example, AP-1, NF-κB, C/EBP and Sp-1.

Given the complexity of ER signalling, as well as the various types of tissue that express ER and its co-factors, it is commonly believed that ER ligands can no longer simply be classified as either pure antagonists or agonists. This view is supported by the findings of Paech et al. (Science 277, 1508-1510, 1997) who have reported that 17β-estradiol activates an AP-1 site in the presence of ERα, but inhibits the same site in the presence of ERβ. In contrast, the ER ligands raloxifene (Eli Lilly & Co.) and tamoxifen and ICI-182,780 (Zeneca Pharmaceuticals) stimulate the AP-1 site through ERβ, but inhibit this site in the presence of ERα.

ERα and ERβ are known to have both overlapping and different tissue distributions, as analysed predominantly by RT-PCR or in-situ hybridisation. Very often tissues express both ERα and ERβ, but the receptors are localised in different cell types.

In summary, although the mechanisms by which the present estrogenic component exerts its favourable effect are as yet unknown, it is evident that said estrogenic component is different from estrogenic substances, such as 17β-estradiol and ethinyl estradiol, in that it exhibits a relatively high affinity for the ERα receptor in comparison to the ERβ receptor. It will also be clear from the above that this specificity may well be responsible for the unexpected efficacy of the present estrogenic component in the treatment or prevention of estrogen-sensitive tumours.

Similarly to SERMs like tamoxifen, the present estrogenic component displays estrogenic effects that enable long term administration without the occurrence of climacteric complaints. Tamoxifen, however, has an undesirable estrogenic effect on uterine tissues and has been associated with endometrial hyperplasia and carcinoma. Long term use of tamoxifen is linked to an increased risk of endometrial cancer, up to a fivefold excess of risk relative to women not treated with tamoxifen therapy. Therefore, application of tamoxifen for long term breast cancer prevention and long term treatment of breast cancer has significant associated risks.

Another disadvantage associated with the tamoxifen in premenopausal women is the risk of ovarian hyperstimulation, leading to excessive secretion of estrogen. It will be evident that the resulting increase in estrogen serum level is highly undesirable in patients with estrogen-sensitive tumours. This is why ovariectomy is commonly applied in premenopausal patients that are treated with tamoxifen. The present estrogenic component does not appear to have such an undesirable impact on uterine tissues, nor does it induce ovarian hyperstimulation, because it actually inhibits follicle growth and ovulation.

Another important benefit of the present estrogenic substances is derived from their relative insensitivity to interactions with other drugs (drug-drug interactions). It is well known that certain drugs may decrease the effectiveness of estrogens and other drugs may enhance their activity, resulting in possible increased side-effects. Similarly estrogens may interfere with the metabolism of other drugs. In general, the effect of other drugs on estrogens is due to interference with the absorption, metabolism or excretion of these estrogens, whereas the effect of estrogens on other drugs is due to competition for metabolic pathways.

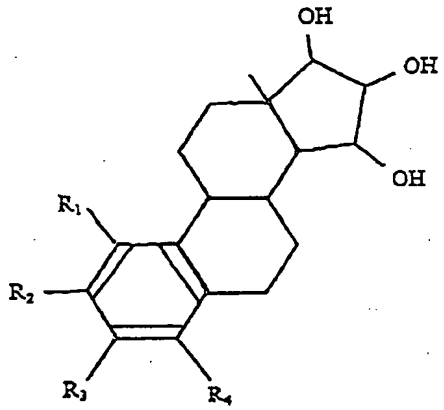
The clinically most significant group of estrogen-drug interactions occurs with drugs that may induce hepatic microsomal enzymes which may decrease estrogen plasma levels below therapeutic level (for example, anticonvulsant agents; phenytoin, primidone, barbiturates, carbamazepine, ethosuximide, and methosuximide; antituberculous drugs such as rifampin; antifungal drugs such as griseofulvin). The present estrogenic substances are less dependent on up- and downregulation of microsomal liver enzymes (e.g. P450's) and also are less sensitive to competition with other P450 substrates. Similarly, they do not interfere significantly in the metabolism of other drugs.

The conjugates of most estrogens, as formed in the liver, are excreted in the bile and may be broken down by gut bacteria in the colon to liberate the active hormone which can then be reabsorbed (enterohepatic recirculation). There are clinical reports that support the view that enterohepatic recirculation of estrogens decreases in women taking antibiotics such as ampicillin, tetracycline, etc. Conjugated forms of the present estrogenic substances are hardly excreted in the bile, meaning that they are substantially insensitive to drugs that do influence the enterohepatic recirculation of other estrogens.

The above observations serve to explain why the estrogenic substances of the invention are particularly suitable for treating or preventing estrogen-sensitive tumours.

DETAILED DESCRIPTION OF THE INVENTION

Accordingly, the present invention relates to a method of treating or preventing estrogen-sensitive tumours in a mammal, said method comprising the administration of a therapeutically effective amount of an estrogenic component to said mammal, wherein the estrogenic component is selected from the group consisting of:
substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms; precursors capable of liberating a substance according to the aforementioned formula when used in the present method; and mixtures of one or more of the aforementioned substances and/or precursors.

As used herein the term "tumour" refers to a new growth of tissue in which the multiplication of cells is uncontrolled and progressive. The term tumour encompasses both malignant and benign tumours.

The term "estrogen-sensitive tumour" refers to a tumour whose formation and growth is stimulated by estrogens, other than the estrogenic components according to the present invention, especially estrogens selected from the group consisting of 17β-estradiol, ethinyl estradiol, as well as precursors and metabolites thereof.

The term "cancer" refers to cells that have undergone a malignant transformation that makes them pathological to the host organism.

The present estrogen substances are distinct from both the biogenic and synthetic estrogens that are commonly applied in pharmaceutical formulations in that the 5 membered ring in the steroid skeleton comprises 3 hydroxyl substituents rather than 0-2. In a particularly preferred embodiment at least one of R_1, R_2, R_3 and R_4 represents a hydroxyl group, meaning that the estrogen substance contains at least 4 hydroxyl groups. Preferably, the estrogenic component applied as the active component in the present composition is a so called biogenic estrogen, i.e. an estrogen that occurs naturally in the human body, a precursor of a biogenic estrogen or a mixture thereof. Because biogenic estrogens are naturally present in the fetal and female body, side-effects are not expected to occur, particularly not if the serum levels resulting from the exogenous administration of such estrogens do not substantially exceed naturally occurring concentrations.

In a preferred embodiment of the present invention the estrogenic substance contains 4 hydroxyl groups. In another preferred embodiment, no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms. Also, in the aforementioned formula, R_1 preferably represents a hydrogen atom. In said formula preferably at least 2, more preferably at least 3 of the groups R_1, R_2, R_3 and R_4 represent a hydrogen atom.

The estrogenic substances according to the formula encompass various enantiomers since the carbon atoms that carry hydroxyl-substituents are chirally active. In one preferred embodiment, the present estrogenic substance is 15α-hydroxy substituted. In another preferred embodiment the substance is 16α-hydroxy substituted. In yet another preferred embodiment, the substance is 17β-hydroxy substituted. Most preferably the estrogenic substances are 15α,16α,17β-trihydroxy substituted. The other chirally active carbon atoms in the steroid skeleton of the present estrogenic components preferably have the same configuration as the corresponding carbon atoms in 17β-estradiol and other biogenic estrogens.

In a preferred embodiment of the present invention R_3 represents a hydroxyl group or an alkoxy group. In another preferred embodiment the groups R_1, R_2 and R_4 represent hydrogen atoms, in which case the substance is 1,3,5 (10)-estratrien-3, 15,16,17-tetrol. A preferred isomer of the latter substance is 1,3,5 (10)-estratrien-3, 15α,16α,17β-tetrol (estetrol).

The invention also encompasses the use of precursors of the estrogen substances that constitute the active component in the present method. These precursors are capable of liberating the aforementioned estrogen substances when used in the present method, e.g. as a result of metabolic conversion. These precursors are preferably selected from the group of

derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue. Typical examples of precursors which can suitably be used in accordance with the invention are esters that can be obtained by reacting the hydroxyl groups of the estrogen substances with substances that contain one or more carboxy (M^+-OOC-) groups, wherein M^+ represents a hydrogen or (akali)metal cation. Hence, in a particularly preferred embodiment, the precursors are derivatives of the estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups in said formula has been substituted by -CO-R, wherein R is a hydrocarbon radical comprising from 1-25 carbon atoms. Preferably R is hydrogen, or an alkyl, alkenyl or aryl radical comprising from 1-20 carbon atoms.

The method according to the present invention may suitably be used to treat mammals such as cattle, pets and particularly humans. The method may be used to treat both females and males (e.g. prostatic hyperplasia), be it that best results are obtained in females. The method may be applied advantageously in premenopausal, perimenopausal and post-menopausal females. Since the present method, unlike SERMs such as tamoxifen, is not associated with the risk of ovarian hyperstimulation, it is especially suited for the treatment of pre- and perimenopausal females. The present method may adavantageously be used to treat estrogen sensitive tumours and also to prevent the occurrence of such tumours.

The present method is particularly effective when the administration is continued for a prolonged period of time. Usually, the method comprises the uninterrupted administration of the estrogenic component during a period of at least 5 days. Preferably the uninterrupted administration is continued for at least 30 days, more preferably for at least 90 days.

The present method may suitably employ enteral or parenteral administration of the estrogenic component. The term "parenteral administration" as used in here encompasses transdermal, intravenous, intranasal, intravaginal, pulmonary, buccal, subcutaneous, intramuscular and intra-uterine administration. The term "enteral administration" includes oral as well as rectal administration.

Preferably the mode of administration is selected from the group consisting of oral, transdermal, intravenous, intranasal, intravaginal, pulmonary, rectal, buccal, subcutaneous, intramuscular or intra-uterine administration. More preferably the mode of administration is selected from the group consisting of oral, transdermal, intravenous, subcutaneous, intranasal, pulmonary and vaginal administration. In a particularly preferred embodiment the present

method employs oral, transdermal, intranasal or subcutaneous administration. Even more preferably the present method employs oral or transdermal administration.

Oral, intravenous, subcutaneous, intramuscular, intranasal, rectal, buccal and pulmonary administration are ideally suited for (at least) once daily administration. Transdermal administration is advantageously applied at frequencies between once a day and once a month. Intravaginal and intra-uterine administrations are advantageously operated at administration frequencies between once weekly and once monthly. Subcutaneous and intramuscular administration may also suitably be done in the form of depot injections at intervals of 1 week to 6 months, preferably at intervals of 4 weeks to 3 months.

For reasons of convenience, the present method preferably utilises administration intervals of 1 day, 1 week or 1 month. Regimens that employ once daily oral, subcutaneous, intravenous or intranasal administration, once weekly transdermal or once monthly intravaginal or subcutaneous administration are particularly preferred.

Although the present method may employ slow release formulations such as the ones described in US 5,340,584, it is preferred not to employ slow release formulations that are effective over an extended period of at least about one month.

Irrespective of the mode of administration, the estrogenic component is preferably administered in an amount effective to achieve a blood serum concentration of at least 1 nanogram per litre, more preferably of at least 10 nanogram per litre, most preferably at least 100 nanogram per litre. Generally the resulting blood serum concentration of the estrogenic component will not exceed 100 μg per litre, preferably it will not exceed 50 μg per litre, more preferably it will not exceed 25 μg per litre.

In a particularly preferred embodiment, the estrogenic component is administered in an amount that clearly exceeds the amount required to maintain serum level of said estrogenic component at a level effective to prevent symptoms of estrogen deficiency, as taught by US 5,340,584. Even more preferably the estrogenic component is administered in an amount sufficient to maintain serum level of said estrogenic component at a level equivalent to a serum level of estradiol of more than 50 pg/ml, most preferably of more than 140 pg/ml.

In accordance with the present method the estrogenic component is usually administered in an amount of less than 1 mg per kg of bodyweight per day, preferably of less than 0.4 mg per kg of bodyweight per day. In order to achieve a significant impact from the administration of the estrogenic component, it is advisable to administer in an amount of at least 1 μg per kg of bodyweight per day. Preferably, the administered amount is at least 5 μg per kg of bodyweight per day.

Oral administration of the active component is preferably done in an amount of less than 400 μg per kg of bodyweight per day, preferably of less than 200 μg per kg of bodyweight per day. In order to achieve a significant impact from the administration of the active component, it is advisable to orally administer in an amount of at least 2 μg per kg of bodyweight per day. Preferably, the orally administered amount is at least 5 μg per kg of bodyweight per day. In the present method, particularly when used in humans, the estrogenic component is usually administered in an average dosage of at least 0.05 mg per day, preferably of at least 0.1 mg per day. The maximum dosage is normally kept below 40 mg per day, preferably below 20 mg per day.

The present method of treatment comprises administering to a mammal in need of such a therapy an effective amount of the estrogenic component. The amount needed to be effective will differ from individual to individual and are determined by factors such as the individual's gender, body weight, route of administration and the efficacy of the particular estrogenic component used.

In the present method, particularly when used in humans, the estrogenic component is usually administered orally in an average dosage of between 0.01 and 20 mg per day, preferably of between 0.05 and 10 mg per day. Similarly, the parenteral dosage preferably is at least 0.05, preferably at least 0.1 mg per day. The average maximum parenteral dosage is normally kept below 40 mg per day, preferably below 20 mg per day.

In a particularly preferred embodiment of the invention the method employs oral administration of the active estrogenic component. The term oral administration as used in here also encompasses oral gavage administration. The inventors have found that, despite its low potency, estetrol and related estrogenic substances may advantageously be administered orally. Although the inventors do not wish to be bound by theory, it is believed that the efficacy of orally administered estetrol-like substances results from the combination of special pharmacokinetic (ADME) and pharmacodynamic properties of these substances.

The inventors have discovered that the oral bioavailability of estetrol-like substances is exceptionally high and that their *in vivo* half-life is considerably longer than that of commonly used biogenic estrogens. Thus, even though estetrol and estetrol-like substances have relatively low estrogenic potency, they may effectively be administered orally because the oral dosages required to achieve the desired effect are similar to those already used for e.g. 17β-estradiol.

Another important advantage of oral administration of estetrol and estetrol-like substances resides in the fact that the hepatic effects of these substances are deemed to be

minimal since they are hardly metabolised during the so called "first pass". The first-pass effect of drugs given orally refers to the process of drug degradation by the liver during a drug's transition from initial ingestion to circulation in the blood stream. After resorption from the intestinal lumen, orally applied active ingredients enter the organism via the liver. This fact is of specific importance for estrogenic agents as the liver is a target organ for estrogens; oral intake of estrogens results in strong estrogenic effects in the liver. Therapeutically equivalent doses of commonly used biogenic estrogens, when applied orally, result in clear responses of hepatic parameters, such as increase of SHBG, CBG and angiotensinogen. These hepatic effects of estrogens are also observed when equine estrogen formulations (so-called conjugated estrogens) are used.

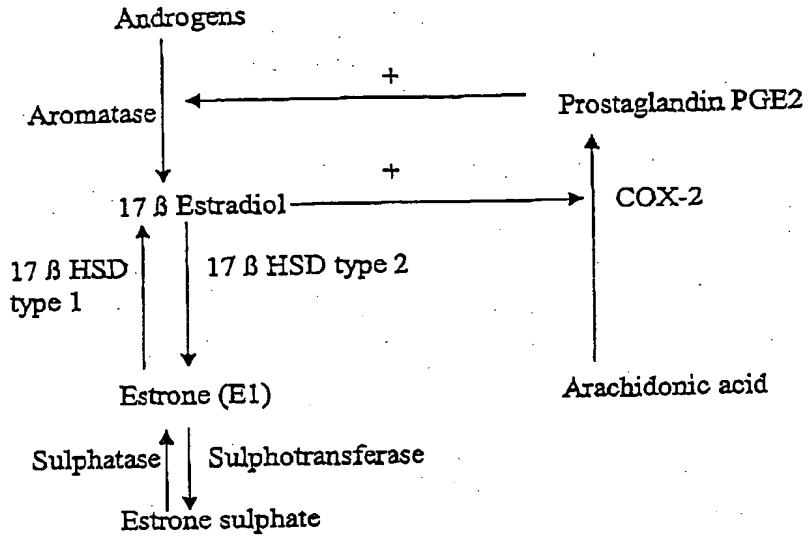
The present method may suitably be used in the (prophylactic) treatment of various estrogen-sensitive tumours, including breast cancer, uterine cancer, ovarian cancer, endometriosis, uterine fibroids, benign prostatic hyperplasia and melanoma. The term "uterine cancer" encompasses endometrial cancer and cervix cancer. The present is method is deemed to be particularly suitable for treating or preventing breast cancer and endometrial cancer. The method of the present invention is most advantageously employed in treating or preventing breast cancer.

In order to further enhance the effectiveness of the present method it may be advisable to co-administer a pharmaceutical component that is capable of suppressing blood serum levels of endogenous estrogens. Preferably one or more of such estrogen suppressants are co-administered in an effective amount to suppress blood serum 17β-estradiol level to below 10 pg/ml, more preferably to below 5 pg/ml, most preferably to below 1 pg/ml.

Examples of estrogen suppressants that may advantageously be co-administered together with the present estrogenic component include progestogens, GnRH analogues, aromatase inhibitors, cyclo-oxygenase 2 (COX-2) inhibitors and 17β-hydroxysteroid dehydrogenase type 1 (17 ß-HSD type 1) inhibitors. Preferably, the present method comprises the co-administration of an estrogen suppressant selected from the aforementioned group of enzyme inhibitors. These enzyme inhibitors offer the advantage that they enable the selective suppression of endogenous estrogen production without directly affecting the production of other steroids and/or gonadotrophins.

In principle, GnRH compositions, as described in US 5,340,584 and US 5,340,585, may also be employed as estrogen suppressants in the present method. Preferably, however, the present method does not employ such a GnRH composition, particularly not if the present method is employed to prevent the occurrence of estrogen-sensitive tumours.

Enzyme inhibitors such as aromatase inhibitors, COX-2 inhibitors and 17β-HSD type 1 inhibitors are capable of blocking biosynthetic pathways that are involved in the endogenous production of the most important endogenous estrogen, i.e. 17β-estradiol. These pathways may be represented as follows:



As is evident from the above diagram, aromatase and 17β-hydroxysteroid dehydrogenase type 1 are key enzymes in the endogenous production of 17β-estradiol. Consequently, the inhibition of aromatase and 17β-hydroxysteroid dehydrogenase type 1 will automatically reduce the endogenous production of 17β-estradiol, which in turn will impair estrogen-induced proliferation.

The diagram also shows that prostaglandin PGE2 is capable of stimulating aromatase activity. Consequently, inhibition of cyclo-oxygenase 2 (COX-2), the enzyme responsible for the endogenous production of PGE2 from arachidonic acid, will automatically cause a reduction of aromatase activity and a corresponding decrease in estrogen-induced proliferation.

Thus it may be concluded that aromatase inhibitors, cyclo-oxygenase 2 (COX-2) inhibitors as well as 17β-hydroxysteroid dehydrogenase type 1 inhibitors may suitably be used to impair endogenous production of estrogens, particularly the endogenous production of 17β-estradiol.

Aromatase is one of the P-450 enzymes. It catalyses the aromatisation of the A ring of the steroid skeleton in the steroid biosynthetic pathway starting from the cleavage of the side chain of cholesterol. To be more precise: aromatase catalyses the conversion of

androstenedione to estrone as well as the conversion of testosterone to estradiol. Hence aromatase is a rate limiting enzyme for the biosynthesis of the latter estrogens.

Aromatase inhibitors are substances capable of inhibiting the catalytic activity of aromatase. In the context of the present invention aromatase inhibitors are substances that may be administered to animals, and especially humans, in non-toxic dosages so as to inhibit estrogen biosynthesis. At present a range of aromatase inhibitors is available and includes substances such as aminoglutethimide, anastrozole, exemestane, vorozole, letrozole, fadrozole, rogletimide, atamestane, formestane, liarozole, YM 511, TZA-2237, CGS 16949A and MEN 11066. Aromatase inhibitors primarily find application in methods of treating breast cancer. It has also been suggested that aromatase inhibitors may be used in the treatment of endometriosis. Takayama et al. (Fertility Sterility 1998; 69(4);709-13) successfully treated one case of an unusually aggressive recurrent postmenopausal endometriosis with an aromatase inhibitor. All existing therapies with aromatase inhibitors are based on oral or intramuscular administration.

Cyclooxygenase (COX), also known as prostaglandin G/H synthase, is a membrane-bound enzyme, responsible for the oxidation of arachidonic acid to prostaglandins, that was first identified over 20 years ago. In the past decade, however, more progress has been made in understanding the role of cyclo-oxygenase enzymes in various pathophysiological conditions. Two cyclo-oxygenase isoforms have been identified and are referred to as COX-1 and COX-2. COX-1 enzyme is constitutively expressed and regulates a number of housekeeping functions such as vascular haemostasis and gastroprotection, whereas COX-2 is inducible (i.e., sites of inflammation) by a number of mediators such as growth factors, cytokines and endotoxins.

Examples of 17β-hydroxysteroid dehydrogenase type 1 inhibitors (17 ß-HSD type 1 inhibitors) include: N-butyl, N-methyl, 9-[3'17'beta-(dihydroxy)-1',3',5'(10')-estratien-16 álpha-yl]-7 bromononamide; N-butyl, N-methyl, 7-[3',17'beta-dihydroxy-1',3',5'(10')-estratiene-6' beta-yl]-7-thiaheptanamide.

In a preferred embodiment, the present method comprises the co-administration of an aromatase inhibitor in an effective amount to suppress endogenous estrogen production. Aromatase inhibitors can suitably be used to achieve a very significant reduction in endogenous estrogen production without serious side-effects. An important side-effect normally associated with aromatase inhibitors, as well as with other suppressants of endogenous estrogen production, i.e. hypoestrogenism, is effectively neutralised by the co-administration of the present estrogenic component.

In a particularly preferred embodiment, the present method comprises the co-administration of a progestogen in an effective amount to suppress endogenous estrogen production. The co-administration of progestogen offers the additional advantage that progestogens are known to inhibit the proliferative effect of estrogens on the endometrium. Although the present estrogenic components, unlike certain SERMs, do not appear to have a pronounced proliferative effect on the endometrium, the co-administration of progestogen may be advisable to rule out any potential risks.

Examples of progestogens which may suitably be used in accordance with the present invention include: progesterone, levonorgestrel, norgestimate, norethisterone, dydrogesterone, drospirenone, 3-beta-hydroxydesogestrel, 3-keto desogestrel (=etonogestrel), 17-deacetyl norgestimate, 19-norprogesterone, acetoxypregnenolone, allylestrenol, anagestone, chlormadinone, cyproterone, demegestone, desogestrel, dienogest, dihydrogesterone, dimethisterone, ethisterone, ethynodiol diacetate, flurogestone acetate, gastrinon, gestodene, gestrinone, hydroxymethylprogesterone, hydroxyprogesterone, lynestrenol (=lynoestrenol), medrogestone, medroxyprogesterone, megestrol, melengestrol, nomegestrol, norethindrone (=norethisterone), norethynodrel, norgestrel (includes d-norgestrel and dl-norgestrel), norgestrienone, normethisterone, progesterone, quingestanol, (17alpha)-17-hydroxy-11-methylene-19-norpregna-4,15-diene-20-yn-3-one, tibolone, trimegestone, algestone acetophenide, nestorone, promegestone, 17-hydroxyprogesterone esters, 19-nor-17hydroxyprogesterone, 17alpha-ethinyl-testosterone, 17alpha-ethinyl-19-nor-testosterone, d-17beta-acetoxy-13beta-ethyl-17alpha-ethinyl-gon-4-en-3-one oxime and precursors of these compounds that are capable of liberating these progestogens *in vivo* when used in the present method. Preferably the progestogen used in the present method is selected from the group consisting of progesterone, desogestrel, etonogestrel, gestodene, dienogest, levonorgestrel, norgestimate, norethisterone, drospirenone, trimegestone, dydrogesterone, precursors of these progestogens and mixtures thereof.

Another aspect of the invention concerns a pharmaceutical composition containing: at least 0.01 mg of an estrogen suppressant selected from the group consisting of aromatase inhibitors, GnRH analogues cyclo-oxygenase 2 (COX-2) inhibitors, 17β-hydroxysteroid dehydrogenase (HSD) type 1 inhibitors and combinations thereof; at least 0.05 mg of the estrogenic component as defined herein before; and pharmaceutically acceptable excipient. In a preferred embodiment, the estrogen suppressant is selected from the group consisting of aromatase inhibitors, COX-2 inhibitors, 17β-HSD type 1 inhibitors and combinations thereof. Most preferably the estrogen suppressant is an aromatase inhibitor.

In a particularly preferred embodiment, the pharmaceutical composition according to invention contains aromatase inhibitor in an amount equivalent to an oral dosage of at least 0.05 mg anastrozole.

The present invention also encompasses a drug delivery system comprising a pharmaceutical composition as defined above, said drug delivery system being selected from the group consisting of an oral dosage unit; an injectable fluid; a suppository; a pessary; a gel; and a cream. In a particularly preferred embodiment said drug delivery system is selected from the group consisting of an oral dosage unit, a suppository, a pessary, a gel and a cream. Most preferably, the drug delivery system is an oral dosage unit.

Yet another aspect of the invention relates to a pharmaceutical kit comprising one or more dosage units containing at least 0.05 mg of the present estrogenic component and a pharmaceutically acceptable excipient; and one or more dosage units containing at least 0.01 mg of an estrogen suppressant selected from the group consisting of GnRH analogues, aromatase inhibitors, cyclo-oxygenase 2 (COX-2) inhibitors, 17β-hydroxysteroid dehydrogenase type 1 inhibitors and combinations thereof, and a pharmaceutically acceptable excipient. Preferably, the dosage units contain the estrogen component in combination with one or more of the aforementioned enzyme inhibitors.

The estrogenic component and the estrogen suppressant can be incorporated in the present kit in the form of separate dosage units. However, it is also possible and indeed very convenient to combine these two components into a single dosage unit.

The pharmaceutical kit preferably contains dosage units for oral, transdermal, intravenous, intranasal, intravaginal, pulmonary, rectal, buccal, subcutaneous, intramuscular and/or intra-uterine administration. More preferably the dosage units are designed for oral, transdermal, intravenous, subcutaneous, intranasal, pulmonary and/or vaginal administration. In a particularly preferred embodiment the kit comprises dosage units for oral, transdermal, intranasal and/or subcutaneous administration. Most preferably, the dosage units are oral dosage units.

The present estrogenic component can suitably be administered in any form of pharmaceutical formulation known in the art. The pharmaceutical formulation can be a solid or semi-solid dosage form such as tablets, capsules, cachets, pellets, pills, powders and granules, as well as fluid dosage forms such as solutions, emulsions, suspensions, ointments, pastes, creams, gels, jellies and foams.

Examples of oral dosage units that may be used in the present method include solid or semi-solid dosage forms such as tablets, capsules, cachets, pellets, pills, powders and

granules. The term "solid or semi-solid dosage form" also encompasses capsules that contain a liquid, e.g. an oil, in which the present estrogenic component is dissolved or dispersed. Tablets and equivalent solid and semi-solid dosage forms can suitably contain materials such as binders (e.g. hydroxypropylmethyl cellulose, polyvinyl pyrrolidine, other cellulosic materials and starch), diluents (e.g. lactose and other sugars, starch, dicalcium phosphate and cellulosic materials), disintegrating agents (e.g. starch polymers and cellulosic materials) and lubricating agents (e.g., stearates and talc).

Suitable transdermal delivery systems include patches, gels, tapes and creams, and can contain excipients such as solubilisers, permeation enhancers (e.g. fatty acids, fatty acid esters, fatty alcohols and amino acids), hydrophilic polymers (e.g. polycarbophil and polyvinyl pyrrolidine) and adhesives and tackifiers (e.g. polyisobutylenes, silicone-based adhesives, acrylates and polybutene).

Examples of transmucosal (notably rectal and intravaginal) delivery systems include patches, tablets, suppositories, pessaries, gels, and creams, and can contain excipients such as solubilizers and enhancers (e.g. propylene glycol, bile salts and amino acids), and other vehicles (e.g. polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethyl cellulose and hyaluronic acid).

Injectable or implantable depot preparations may take the form of injectable fluids and implantation tablets. Suitable fluid carrier components are physiologically compatible diluents wherein the active agents can be dissolved, suspended. An example of a diluent is water, with or without addition of electrolyte salts or thickeners. Thus, the depot formulation can be, for example, an aqueous microcrystalline suspension. Oils are particularly suitable as diluents, with or without the addition of a solubiliser, of a surfactant, or of a suspension or emulsifying agent. Examples of suitable oils include arachidis oil, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil, and sesame oil. Examples of solubilisers include benzyl alcohol and benzyl benzoate. Depot preparations offer the advantage that a single injection or implantation suffices for one or several months. Duration of the depot effect depends the nature of the estrogenic component (the ester precursors being preferred as they display a slower release), the amount of the estrogenic component as well as on the type of carrier substance that releases the active agent. Generally, the duration will be in the range of 10-30 days, but longer or shorter times can also be achieved.

Other delivery systems that can be used for administering the estrogenic components of the invention include intranasal and pulmonary delivery systems such as sprays and microparticles.

The invention is further illustrated by the following examples:

EXAMPLES

Example 1

Established competitive steroid binding assays were used to determine the relative binding affinity of estetrol (E4), as compared to 17α-ethinylestradiol(EE) and 17β-estradiol (E2), to human Estrogen Receptor (ER) α- and β-forms.

The method employed was adapted from the scientific literature and described in detail by Osbourn et al. (1993, Biochemistry, 32, 6229-6236). Recombinant human ERα and ERβ proteins were purified from transfected Sf9-cells. The *in vitro* assays involved the use of either ERα or ERβ proteins and [^{3}H]E2, at a fixed concentration of 0.5 nM, as the labeled ligand. Recombinant human ERα or ERβ proteins were dissolved in binding buffer (10 mM Tris-HCL, pH 7.5, 10% glycerol, 1 mM DTT, 1 mg/ml BSA) and duplicate aliquots were then incubated with [^{3}H]E2 at a final concentration of 0.5 nM, together with a vehicle control (0.4% DMSO), or the same amount of vehicle containing increasing concentrations of unlabeled steroid ligands as competitors. After incubation for 2 h at 25^{0}C, the unbound ligands were removed and the amounts of [^{3}H]E2 bound to either ERα or ERβ proteins were measured. The average amounts of [^{3}H]E2 bound to either ERα or ERβ proteins at each concentration of competitor were used to make inhibition curves. IC50 values were subsequently determined by a non-linear, least squares regression analysis. Inhibition constants (Ki) were calculated using the equation of Cheng and Prusoff (Cheng et al., 1973, Biochem. Pharmacol., 22, 3099-3108), using the measured IC50 of the tested compounds, the concentration of radioligand employed in the assay, and the historical values for the Kd of the radioligand, which were established as 0.2 nM and 0.13 nM for ERα and ERβ, respectively. Biochemical assay results for E4 are presented as the percent inhibition of specific binding in three separate experiments (Table 1). For comparision of binding affinities of E4, EE and E2 to human ERα and ERβ proteins, experimentally observed Ki values are shown in Table 2. As compared to EE and E2, E4 demonstrates a unique binding profile with a strong preference (400%) for binding to the ERα protein (Table 2). In contrast, Ki values for ERβ protein are more pronounced for EE and E2 steroid ligands (Table 2).

Table 1: Percent inhibition of specific binding to ERα and ERβ proteins using E4 as unlabeled steroid ligand and 0.5 nM [3H] E2 as labeled competitor. Results of three separate experiments are shown.

E4 final concentration	Percent inhibition of specific binding in					
	ERα steroid binding assay			ERβ steroid binding assay		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
1 μM	98	nd	Nd	87	90	95
0.3 μM	92	94	101	74	74	77
0.1 μM	83	85	86	56	54	50
0.03 μM	64	66	63	19	25	30
10 nM	43	32	28	nd	nd	nd
3 nM	26	17	11	nd	nd	nd

nd: not determined

Table 2: Experimentally determined inhibition constants (Ki) for estetrol (E4), 17α-ethinylestradiol (EE) and 17β-estradiol (E2), to human ERα and ERβ proteins. Relative preference for binding to ERα protein is also shown.

Steroid ligands	Ki ERα (nM)	Ki ERβ (nM)	Relative ERα/ERβ preference(%)
EE	0.23	0.025	11
E2	0.21	0.015	7
E4	4.9	19	400

Example 2

To determine the bioavailability and elimination half-life of estetrol after oral dosing in humans a single rising dosing study was performed in healthy postmenopausal volunteers. Volunteers (n=6) were randomly assigned to 0.1, 1 or10 mg estetrol and blood samples (18 per volunteer) were obtained over a period of 72 hours.

After thawing the plasma samples, liquid-liquid extraction (hexane and diethyl ether) was employed to prepare the estetrol-containing plasma samples for HPLC analysis (Perkin Elmer 200) and tandem mass spectrometry using a PE Sciex 4000 tandem mass spectrometer and APCI interface. With each sample batch, a calibration curve with 6 calibrators was

recorded. The calibration curve was calculated using linear regression (correlation coefficient > 0.98), which permitted quantitation of plasma concentrations.

Good tolerability was observed when increasing the oral estetrol dose from 0.1 to 1 and further to 10 mg. AUC values demonstrated good dose-linearity, indicating that, over the entire dose range, orally administered estetrol was well absorbed. Interestingly, estetrol demonstrated a long elimination half-life of more than 20 hours, i.e.20-50 hours in human postmenopausal subjects.

Example 3

In order to assess the anti-tumour efficacy of the estrogenic substances of the present invention, estetrol was tested in the 7, 12-dimethyl-benz(a)anthracene (DMBA)-induced tumour model in rats. This model, originally developed by Huggins et al.,1961 (Nature,19, 204-207), has been widely used and is a generally accepted model with predictive value for anti-tumour agents in humans. The growth of the DMBA-induced tumours is dependent on endogenously produced estradiol or exogenously administered estrogens and prolactin (Sylvester et al., 1982, Cancer Research, 42, 4943-4947). Ovariectomy (Hollingsworth et al., 1998, Breast Cancer Research and Treatment, 47, 63-70), androgens (Dauvois et al., 1989, Breast Cancer Treatment, 14, 299-306), tamoxifen (Hollingsworth et al., 1998, Breast Cancer Research and Treatment, 47, 63-70), progestogens (Kelly et al. 1979, Eur. J. Cancer, 15, 1243-1251; Russo et al., 1987, Lab. Invest. 57, 112-137) and GnRH analogues (Hollingsworth et al., 1998, Breast Cancer Research and Treatment, 47, 63-70) all have been shown to be effective anti-tumour treatments in the DMBA model.

Eighty-four female Sprague-Dawley rats (Harlan, The Netherlands) were group housed, maintained in a 12-hr light/dark environment, and fed a Soya Free Diet (SDS England) and water ad libitum. Animals were weighed on a weekly basis. One week prior to induction of mammary carcinoma, 12 animals (aged 43 days) were surgically castrated via removal of the ovaries. At the age of 50 days, all animals were administered a single oral dose of 16 mg DMBA to induce tumour development. Animals were subsequently allocated to one of seven groups (n=12), receiving placebo or treatment as follows:.

- *Group 1* animals received placebo oral treatment with 3.0 ml/kg/day vehicle (20% wt/vol solution of hydroxypropyl-beta-cyclodextrin in water);
- *Group 2* surgically castrated animals received placebo treatment with 3.0 ml/kg/day vehicle;

- *Group 3* animals received the anti-estrogen tamoxifen given orally at a single daily dose of 3 mg/kg;
- *Group 4* animals received ethinylestradiol (EE) orally at a single daily dose of 0.025 mg/kg;
- *Group 5* animals received ethinylestradiol (EE) orally at a single daily dose of 0.125 mg/kg;
- *Group 6* animals received estetrol (E4) orally at a single daily dose of 0.5 mg/kg; and
- *Group 7* animals received estetrol (E4) orally at a single daily dose of 2.5 mg/kg.

The doses of EE and E4 were based on data from previous studies, showing equipotency of 0.025 mg/kg/day EE and 0.5 mg/kg/day E4 in agonistic models of preventing bone resorption, prevention of hot flushing and vaginal cornification. Similarly, the doses of 0.125 mg/kg/day EE and 2.5 mg/kg/day E4 showed equipotency in *in vivo* estrogenicity in preventing bone resorption, prevention of hot flushing and vaginal cornification.

During the treatment period of 8 weeks, the emergence of palpable tumours and number of tumours were determined weekly. At 8 weeks, at necropsy, final measurements were taken. The number of tumours at necropsy are depicted in figure 1

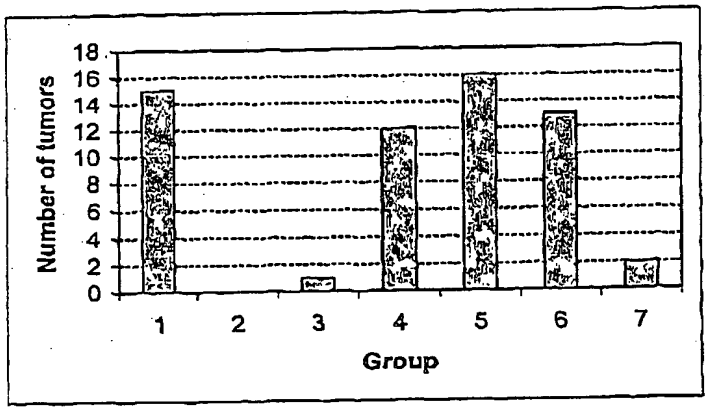


Figure 1. Number of mammary tumours per treatment group (n=12).

Group 1 oral treatment with 3.0 ml/kg/day vehicle;

Group 2 surgically castrated animals receiving placebo treatment with 3.0 ml/kg/day vehicle;

Group 3 tamoxifen 3 mg/kg/day orally;

Group 4 ethinylestradiol (EE) 0.025 mg/kg/day orally;

Group 5 EE 0.125 mg/kg/day orally;

Group 6 estetrol (E4) 0.5 mg/kg/day orally;

Group 7 E4 2.5 mg/kg/day orally.

As is clearly demonstrated by the absence of tumours in the ovariectomized animals (group 2), development of DMBA-induced mammary tumours is estrogen-dependent. As expected, also tamoxifen showed anti-tumour properties by inhibiting the development of mammary tumours in this model. Surprisingly, and in contrast to the effect seen with the 0.125 mg/kg/day dose of EE, E4 at an equipotent agonistic dose of 2.5 mg/kg/day markedly suppressed mammary tumour development. Furthermore, this particular dose of E4 was as effective as tamoxifen in preventing growth of DMBA-induced tumours.

Example 4

Estetrol and tamoxifen were subsequently tested in a second DMBA trial in rats to evaluate the dose-response relationships in preventing rats from developing mammary tumours. The experimental procedure as set forth in example 3 was used as a prevention study to treat the animals (12 animals per group) for 8 consecutive weeks after tumour induction with oral dosages of either estetrol or tamoxifen. DMBA-exposed rats were randomly assigned to treatment groups, receiving oral treatment as follows:

- *Group 1* animals received placebo oral treatment in the form of a single daily dose of 3.0 ml/kg vehicle (20% wt/vol solution of hydroxypropyl-beta-cyclodextrin in water);
- *Group 2* animals received tamoxifen orally at a single daily dose of 1 mg/kg;
- *Group 3* animals received tamoxifen orally at a single daily dose of 2 mg/kg;
- *Group 4* animals received tamoxifen orally at a single daily dose of 3 mg/kg;
- *Group 5* animals received estetrol orally at a single daily dose of 0.5 mg/kg;
- *Group 6* animals received estetrol orally at a single daily dose of 1.0 mg/kg;
- *Group 7* animals received estetrol orally at a single daily dose of 1.5 mg/kg;
- *Group 8* animals received estetrol orally at a single daily dose of 2.0 mg/kg;
- *Group 9* animals received estetrol orally at a single daily dose of 2.5 mg/kg; and
- *Group 10* animals received estetrol orally at a single daily dose of 3.0 mg/kg

During the treatment period of 8 weeks, the emergence of palpable tumours and number of tumours were determined weekly. The number of mammary tumours at necropsy is depicted in figure 2. As expected, tamoxifen showed an anti-proliferative effect on development of mammary tumours in this prevention study. In none of the tamoxifen groups (1, 2, and 3 mg/kg/day) palpable tumours developed. Oral estetrol treatment (0.5-3.0 mg/kg/day) also showed a dose-dependent inhibition of mammary tumour formation, further confirming its anti-proliferative effect on tumor growth. Furthermore, and as observed for

tamoxifen, treatment with 2.5 and 3.0 mg/kg/day estetrol completely protected rats from developing tumours.

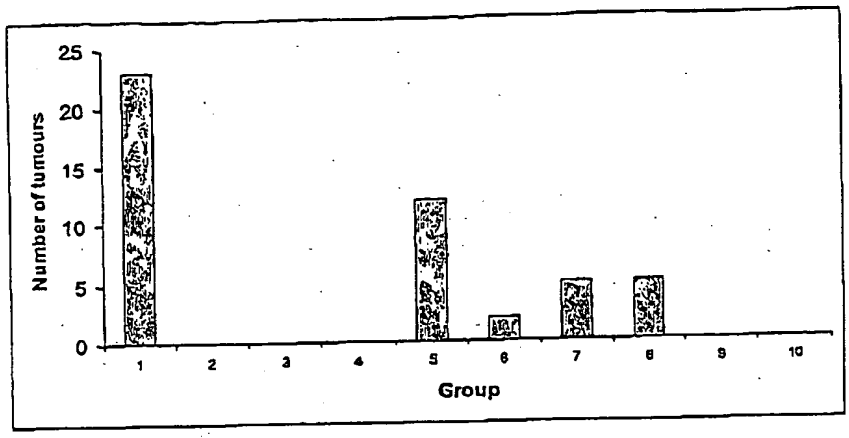


Figure 2. Number of mammary tumours per treatment group (n=12).
Group 1 oral treatment with 3.0 ml/kg/day vehicle;
Group 2 tamoxifen 1 mg/kg/day orally;
Group 3 tamoxifen 2 mg/kg/day orally;
Group 4 tamoxifen 3 mg/kg/day orally;
Group 5 estetrol (E4) 0.5 mg/kg/day orally;
Group 6 E4 1.0 mg/kg/day orally;
Group 7 E4 1.5 mg/kg/day orally;
Group 8 E4 2.0 mg/kg/day orally;
Group 9 E4 2.5 mg/kg/day orally;
Group 10 E4 3.0 mg/kg/day orally.

Example 5

In order to assess the efficacy of estetrol to reduce the number and size of pre-existing mammary tumours, estetrol was tested in a modified version (therapeutic design) of the 7, 12-dimethyl-benz(a)anthracene (DMBA)-induced tumour model in rats. As set forth in example 3, female Sprague-Dawley rats were given 16 mg DMBA at the age of 50 days. Mammary tumour development was allowed to proceed until week 8 after DMBA treatment. Animals were subsequently allocated to one of six groups, receiving 4 weeks daily oral treatment with placebo, tamoxifen or estetrol as follows:

- *Group 1* animals received placebo treatment with a single daily dose of 3.0 ml/kg vehicle (20% wt/vol solution of hydroxypropyl-beta-cyclodextrin in water);

- *Group 2* animals were surgically castrated and received placebo treatment with 3.0 ml/kg/day vehicle;
- *Group 3* animals received tamoxifen at a dose of 1 mg/kg;
- *Group 4* animals received estetrol at a dose of 1.0 mg/kg;
- *Group 5* animals received estetrol at a dose of 3.0 mg/kg;
- *Group 6* animals received estetrol at a dose of 10.0 mg/kg.

The oral doses of estetrol and tamoxifen were selected on the basis of previous findings showing partial or complete suppression of mammary tumour development in a preventive mode of the DMBA model (see example 3 and example 4).

During therapy, the progression or disappearance of palpable mammary tumours and the size of the tumours were determined weekly. At necropsy, tumours were counted, measured and the change from baseline at the start of treatment was calculated.

In vehicle treated animals (n=9) tumour count increased steeply from 16 at the start of treatment to 35 after 4 weeks of therapy. Ovariectomized rats (n=8) showed a 53% decrease in tumour count from 15 at the start of treatment to 7 at necropsy. Despite its efficacy in preventing mammary tumour development immediately after tumour induction with DMBA, tamoxifen at a dose of 1 mg/kg/day did not prevent a further increase in tumour number when administered 8 weeks after DMBA induction. In tamoxifen-treated rats (n=8) the tumour number further increased from 15 at the start of treatment to 19 at necropsy. Interestingly, estetrol dose-dependently reduced the number of pre-existing mammary tumours during the 4 week therapeutic trial. In rats treated with estetrol at a dose of 1 mg/kg/day (n=9), estetrol was marginally effective as indicated by an increase from 16 tumours at the start of treatment to 23 at necropsy. In rats treated with 3 mg/kg/day estetrol (n=9) tumour counts were slightly reduced from 16 at the start of treatment to 15 at necropsy. Furthermore, in rats treated with 10 mg/kg/day estetrol (n=10) tumour number declined from 18 at the start of treatment to 7 at necropsy.

Hence, from the analysis of the net disappearance of mammary tumours it is evident that the efficacy of estetrol is comparable to ovariectomy. Tamoxifen, at an effective dose to prevent the outgrowth of mammary tumours, was ineffective at later stages in the model to counteract the further development and progression mammary tumours. By expressing the tumour counts as a percentage change from baseline at the start of treatment (figure 3), the strong therapeutic efficacy of estetrol becomes clearly evident.

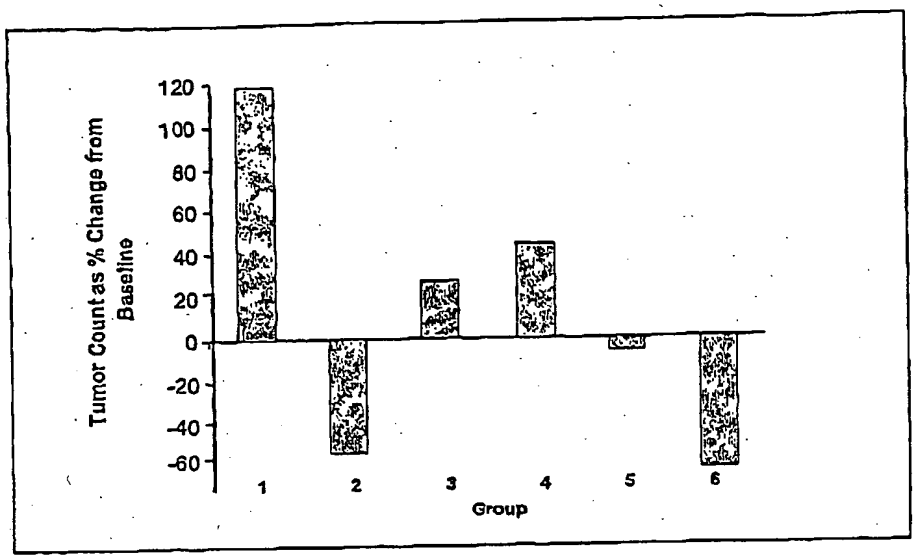


Figure 3. Responsiveness of pre-existing mammary tumours to ovariectomy or 4 weeks oral treatment with tamoxifen or estetrol.

Group 1 oral treatment with 3.0 ml/kg/day vehicle;

Group 2 surgically castrated animals receiving placebo treatment with 3.0 ml/kg/day vehicle;

Group3 tamoxifen 1 mg/kg/day orally;

Group 4 estetrol 1 mg/kg/day orally;

Group 5 estetrol 3 mg/kg/day orally;

Group 6 estetrol 10 mg/kg/day orally.

Similarly, by expressing the tumour sizes as percentage change from baseline, estetrol treatment (like ovariectomy) was shown to be effective in causing a dose dependent pronounced tumour size reduction as a net group effect (figure 4). Although reduction of tumour size was observed for individually treated rats, the net balance of treating the animals with tamoxifen was less favourable, showing an increase in tumour size as net group effect.

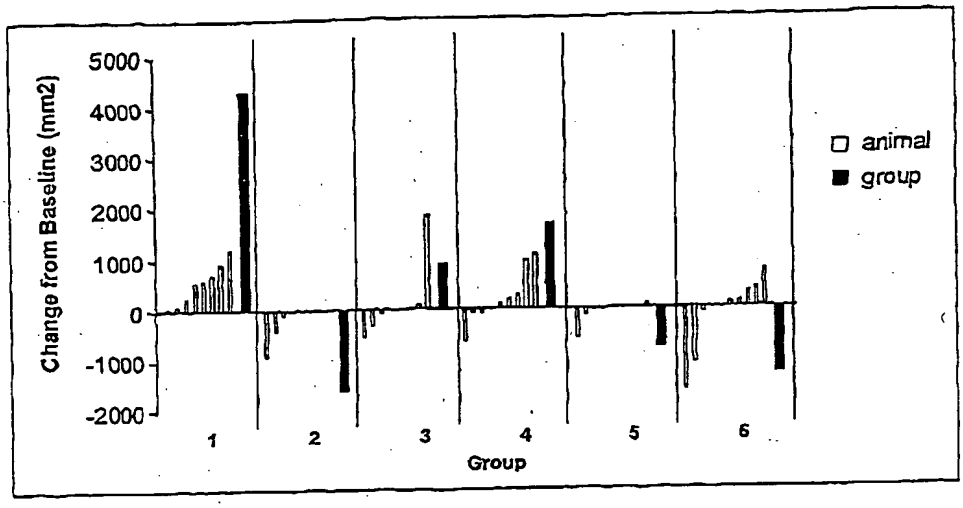


Figure 4. Mammary tumour load per animal (white bars) and per group (black bars) in response to ovariectomy or 4 weeks oral treatment with tamoxifen or estetrol.

Group 1 oral treatment with 3.0 ml/kg/day vehicle;

Group 2 surgically castrated animals receiving placebo treatment with 3.0 ml/kg/day vehicle;

Group3 tamoxifen 1 mg/kg/day orally;

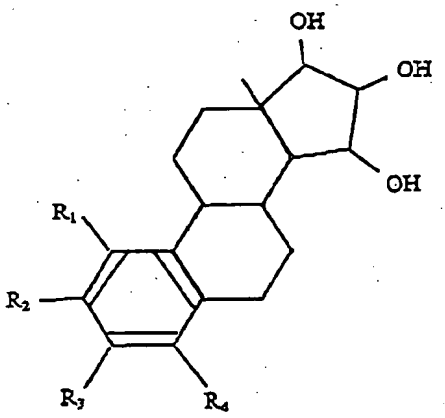
Group 4 estetrol 1 mg/kg/day orally;

Group 5 estetrol 3 mg/kg/day orally;

Group 6 estetrol 10 mg/kg/day orally.

CLAIMS

1. Use of an estrogenic component selected from the group consisting of:
substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms;
precursors capable of liberating a substance according to the aforementioned formula when used in the present method; and
mixtures of one or more of the aforementioned substances and/or precursors;
in the manufacture of a pharmaceutical composition for use in a method of treating or preventing estrogen-sensitive tumours in a mammal, said method comprising the administration of a therapeutically effective amount of the estrogenic component to said mammal.

2. Use according to claim 1, wherein no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms;

3. Use according to claim 1 or 2, wherein R_3 represents a hydroxyl group or an alkoxy group.

4. Use according to any one of claims 1-3, wherein at least 3 of the groups R_1, R_2, R_3 and R_4 represent hydrogen atoms.

5. Use according to any one of claims 1-4, wherein the precursors capable of liberating the estrogenic substance are derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue.

6. Use according to any one of claims 1-5 , wherein the method comprises the uninterrupted administration of the estrogenic component during a period of at least 5 days, preferably of at least 30 days.

7. Use according to any one of claims 1-6, wherein the method comprises oral, transdermal, intravenous or subcutaneous administration of the estrogenic component.

8. Use according to claim 7, wherein the method comprises oral administration.

9. Use according to any one of claims 1-8, wherein the estrogenic component is administered in an amount of at least 1 μg per kg of bodyweight per day, preferably of at least 5 μg per kg of bodyweight per day.

10. Use according to any one of claims 1-9, wherein the estrogen-sensitive tumours are selected from the group consisting of breast cancer, uterine cancer, ovarian cancer, endometriosis, uterine fibroids, benign prostatic hyperplasia and melanoma.

11. Use according to claim 10 wherein the estrogen-sensitive tumours are selected from the group consisting of breast cancer and uterine cancer.

12. Use according to any one of claims 1-11, wherein the method comprises co-administration of an estrogen suppressant in an amount effective to suppress the endogenous estrogen production, wherein said estrogen suppressant is selected from the group consisting of progestogens, GnRH analogues, aromatase inhibitors, cyclo-oxygenase 2 (COX-2) inhibitors, 17β-hydroxysteroid dehydrogenase type 1 inhibitors and combinations thereof.

13. Use according to claim 11, wherein the estrogen suppressant is co-administered in an effective amount to suppress blood serum 17β-estradiol level to below 10 pg/ml, more preferably to below 5 pg/ml, most preferably to below 1 pg/ml

14. Use according to claim 11 or 12, wherein the method comprises co-administration of a progestogen

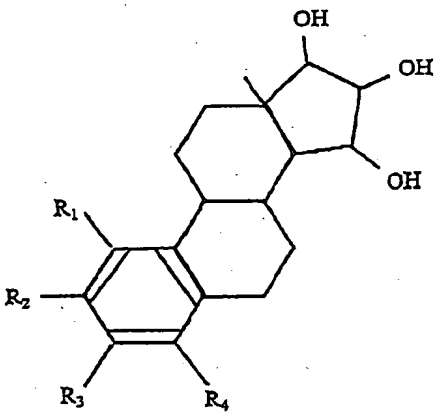
15. Use according to claim 11 or 12, wherein the method comprises co-administration of an aromatase inhibitor.

16. A pharmaceutical composition containing:

a. at least 0.01 mg of an estrogen suppressant selected from the group consisting of aromatase inhibitors, GnRH analogues and combinations thereof;

b. at least 0.05 mg of an estrogenic component selected from the group consisting of:

substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms;

precursors capable of liberating a substance according to the aforementioned formula when used in the present method; and mixtures of one or more of the aforementioned substances and/or precursors; and

c. pharmaceutically acceptable excipient.

17. The pharmaceutical composition according to claim 16, wherein no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms.

18. The pharmaceutical composition according to claim 16 or 17, wherein R_3 represents a hydroxyl group or an alkoxy group.

19. The pharmaceutical composition according to any one of claims 16-18, wherein at least 3 of the groups R_1, R_2, R_3 and R_4 represent hydrogen atoms.

20. The pharmaceutical composition according to any one of claims 16-19, wherein the precursors capable of liberating the estrogenic substance are derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue.

21. The pharmaceutical composition according to any one of claims 16-20, wherein the composition contains aromatase inhibitor in an amount equivalent to an oral dosage of at least 0.05 mg anastrozole.

22. A drug delivery system comprising a pharmaceutical composition according to any one of claims 16-21, said drug delivery system being selected from the group consisting of an oral dosage unit; an injectable fluid; a suppository; a pessary; a gel; and a cream.

23. A pharmaceutical kit comprising one or more dosage units containing at least 0.05 mg of the estrogenic component as defined in claim 1 and a pharmaceutically acceptable excipient; and one or more dosage units containing at least 0.01 mg of an estrogen suppressant selected from the group consisting of GnRH analogues, aromatase inhibitors, cyclo-oxygenase 2 (COX-2) inhibitors, 17β-hydroxysteroid dehydrogenase type 1 inhibitors and combinations thereof, and a pharmaceutically acceptable excipient.

24. The pharmaceutical kit according to claim 23, wherein the dosage units are oral dosage units.

INTERNATIONAL SEARCH REPORT

Int/ plication No
PCT/NL 03/00513

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/565 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 340 585 A (PIKE MALCOLM C ET AL) 23 August 1994 (1994-08-23) column 12, line 25-44; table 1 claims 1,3,4,13 column 13, line 67 column 15, line 1 ---	1-4, 6-10, 12-19, 22-24
X	WO 94 26207 A (UNIV SOUTHERN CALIFORNIA) 24 November 1994 (1994-11-24) page 17, line 5-30; table 1 claims 1,4,13 page 19, last line --- -/—	1-4, 6-10, 12-19, 22-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2003	07/10/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Veronese, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 340 584 A (PIKE MALCOLM C ET AL) 23 August 1994 (1994-08-23) column 14, line 11-43; claims 1,4,16 table 1 column 16, line 11 column 17, line 30 column 7, line 13	1-4, 6-10, 12-19, 22-24
P,X	WO 02 100877 A (SOUTHWEST FOUND BIOMED RES) 19 December 2002 (2002-12-19) page 15, line 15 -page 16-30; claims 28,73,80,81	1,3,5-10
P,X	WO 02 094276 A (BUNSCHOTEN EVERT JOHANNES ;COELINGH BENNINK HERMAN JAN TI (NL); PA) 28 November 2002 (2002-11-28) *See page 10, lines 18-19: breast cancer* claims 1-15 See claim 5, cancer *	1-11
X	US 4 937 238 A (LEMON HENRY M) 26 June 1990 (1990-06-26) column 1, line 63 -column 2 column 3, line 19-25	1-4,6-11
X	LIPPERT CAROLINE ET AL: "The effects of A-ring and D-ring metabolites of estradiol on the proliferation of vascular endothelial cells" LIFE SCIENCES, PERGAMON PRESS, OXFORD, GB, vol. 67, no. 13, 18 August 2000 (2000-08-18), pages 1653-1658, XP002200988 ISSN: 0024-3205 See table 1, estetrol abstract page 1657, last paragraph	1-10
A	NAMBARA T ET AL: "SYNTHESIS OF ESTEROL MONOGLUCURONIDES" STEROIDS, BUTTERWORTH-HEINEMANN, STONEHAM, MA, US, vol. 27, no. 1, January 1976 (1976-01), pages 111-122, XP009004815 ISSN: 0039-128X * See charts 1-3 *	5
A	US 5 468 736 A (HODGEN GARY D) 21 November 1995 (1995-11-21) column 3, line 5-10 example 3 claims	12-19

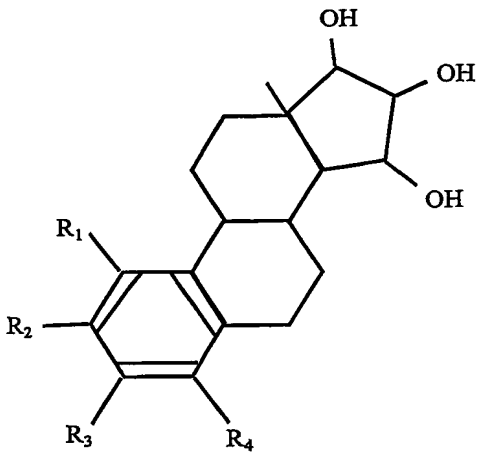
-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MUECK A O ET AL: "ANGIOGENETIC AND ANTI-ANGIOGENETIC EFFECTS OF ESTRADIOL AND ITS METABOLITES" JOURNAL OF CLINICAL AND BASIC CARDIOLOGY, XX, AU, vol. 4, 2001, pages 153-155, XP008004903 ISSN: 1561-2775 * See table 1, estetrol * page 154, column 1	1
A	BAYARD F (REPRINT) ET AL: "EFFECTS OF ESTRADIOL, ESTRIOL, ESTETROL AND ESTRONE ON A STRAIN OF HUMAN-BREAST CANCER -CELLS IN CULTURE (MCF -7)" ANNALES D ENDOCRINOLOGIE, (1980) VOL. 41, NO. 5, PP. C26., 1880, XP001121532 CTR HOSP UNIV TOULOUSE RANGUEIL, INSERM, U168, F-31054 TOULOUSE, FRANCE the whole document	1

CLAIMS

1. Use of an estrogenic component selected from the group consisting of:
substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms;
precursors capable of liberating a substance according to the aforementioned formula when used in the present method; and
mixtures of one or more of the aforementioned substances and/or precursors;
in the manufacture of a pharmaceutical composition for use in a method of treating or preventing estrogen-sensitive tumours in a mammal, said method comprising the administration of a therapeutically effective amount of the estrogenic component to said mammal.

2. Use according to claim 1, wherein no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms;

3. Use according to claim 1 or 2, wherein R_3 represents a hydroxyl group or an alkoxy group.

4. Use according to any one of claims 1-3, wherein at least 3 of the groups R_1, R_2, R_3 and R_4 represent hydrogen atoms.

5. Use according to any one of claims 1-4, wherein the precursors capable of liberating the estrogenic substance are derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue.

6. Use according to any one of claims 1-5 , wherein the method comprises the uninterrupted administration of the estrogenic component during a period of at least 5 days, preferably of at least 30 days.

7. Use according to any one of claims 1-6, wherein the method comprises oral, transdermal, intravenous or subcutaneous administration of the estrogenic component.

8. Use according to claim 7, wherein the method comprises oral administration.

9. Use according to any one of claims 1-8, wherein the estrogenic component is administered in an amount of at least 1 μg per kg of bodyweight per day, preferably of at least 5 μg per kg of bodyweight per day.

10. Use according to any one of claims 1-9, wherein the estrogen-sensitive tumours are selected from the group consisting of breast cancer, uterine cancer, ovarian cancer, endometriosis, uterine fibroids, benign prostatic hyperplasia and melanoma.

11. Use according to claim 10 wherein the estrogen-sensitive tumours are selected from the group consisting of breast cancer and uterine cancer.

12. Use according to any one of claims 1-11, wherein the method comprises co-administration of an estrogen suppressant in an amount effective to suppress the endogenous estrogen production, wherein said estrogen suppressant is selected from the group consisting of progestogens, GnRH analogues, aromatase inhibitors, cyclo-oxygenase 2 (COX-2) inhibitors, 17β-hydroxysteroid dehydrogenase type 1 inhibitors and combinations thereof.

13. Use according to claim 11, wherein the estrogen suppressant is co-administered in an effective amount to suppress blood serum 17β-estradiol level to below 10 pg/ml, more preferably to below 5 pg/ml, most preferably to below 1 pg/ml

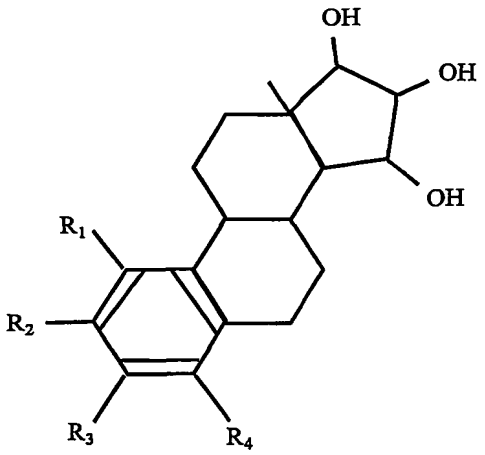
14. Use according to claim 11 or 12, wherein the method comprises co-administration of a progestogen

15. Use according to claim 11 or 12, wherein the method comprises co-administration of an aromatase inhibitor.

16. A pharmaceutical composition containing:

a. at least 0.01 mg of an estrogen suppressant selected from the group consisting of aromatase inhibitors, GnRH analogues and combinations thereof;

b. at least 0.05 mg of an estrogenic component selected from the group consisting of: substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms;

precursors capable of liberating a substance according to the aforementioned formula when used in the present method; and mixtures of one or more of the aforementioned substances and/or precursors; and

c. pharmaceutically acceptable excipient.

17. The pharmaceutical composition according to claim 16, wherein no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms.

18. The pharmaceutical composition according to claim 16 or 17, wherein R_3 represents a hydroxyl group or an alkoxy group.

19. The pharmaceutical composition according to any one of claims 16-18, wherein at least 3 of the groups R_1, R_2, R_3 and R_4 represent hydrogen atoms.

20. The pharmaceutical composition according to any one of claims 16-19, wherein the precursors capable of liberating the estrogenic substance are derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue.

21. The pharmaceutical composition according to any one of claims 16-20, wherein the composition contains aromatase inhibitor in an amount equivalent to an oral dosage of at least 0.05 mg anastrozole.

22. A drug delivery system comprising a pharmaceutical composition according to any one of claims 16-21, said drug delivery system being selected from the group consisting of an oral dosage unit; an injectable fluid; a suppository; a pessary; a gel; and a cream.

23. A pharmaceutical kit comprising one or more dosage units containing at least 0.05 mg of the estrogenic component as defined in claim 1 and a pharmaceutically acceptable excipient; and one or more dosage units containing at least 0.01 mg of an estrogen suppressant selected from the group consisting of GnRH analogues, aromatase inhibitors, cyclo-oxygenase 2 (COX-2) inhibitors, 17β-hydroxysteroid dehydrogenase type 1 inhibitors and combinations thereof, and a pharmaceutically acceptable excipient.

24. The pharmaceutical kit according to claim 23, wherein the dosage units are oral dosage units.

IPER WITH ANNEXES

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Van Westenbrugge, A.
NEDERLANDSCH OCTROOIBUREAU
P.O.Box 29720
Scheveningseweg 82
NL-2502 LS The Hague
PAYS-BAS

preliminary examination report
rapport d'examen préliminaire:
28-11-04
termijn om/délai en regional fase:
12-1-05

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 28.10.2004
Applicant's or agent's file reference P045194PCT DBO/jdo	IMPORTANT NOTIFICATION
International application No. PCT/NL 03/00513	International filing date *(day/month/year)* 11.07.2003 / Priority date *(day/month/year)* 12.07.2002
Applicant PANTARHEI BIOSCIENCE B.V. et al.	

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Senkel, H Tel. +49 89 2399-8071

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P045194PCT DBO/jdo	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/NL 03/00513	International filing date *(day/month/year)* 11.07.2003	Priority date *(day/month/year)* 12.07.2002
International Patent Classification (IPC) or both national classification and IPC A61K31/565		
Applicant PANTARHEI BIOSCIENCE B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.01.2004	28.10.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Veronese, A Telephone No. +49 89 2399-7824

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL 03/00513

I. Basis of the report

1. With regard to the elements of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-27 as originally filed

Claims, Numbers

1-21 received on 15.09.2004 with letter of 15.09.2004

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL 03/00513

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-21
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/NL 03/00513

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents; unless otherwise indicated, reference is made to the relevant passages emphasized in the Search Report.

D1: US-A-5 340 585 (PIKE MALCOLM C ET AL) 23 August 1994
D2: WO 94 26207 A (UNIV SOUTHERN CALIFORNIA) 24 November 1994
D3: US-A-5 340 584 (PIKE MALCOLM C ET AL) 23 August 1994
D4: WO 02 100877 A (SOUTHWEST FOUND BIOMED RES) 19 December 2002
D5: WO 02 094276 A (BUNSCHOTEN EVERT JOHANNES); 28 November 2002
D6: US-A-4 937 238 (LEMON HENRY M) 26 June 1990
D7: LIPPERT CAROLINE ET AL: "The effects of A-ring and D-ring metabolites of estradiol on the proliferation of vascular endothelial cells" LIFE SCIENCES, PERGAMON PRESS, OXFORD, GB, vol. 67(13), 18 August 2000 pg 1653-1658.
D8: NAMBARA T ET AL: "SYNTHESIS OF ESTEROL MONOGLUCURONIDES" STEROIDS, MA, US, vol. 27(1), January 1976, pages 111-122, XP009004815
D9 US 5 468 736 A (HODGEN GARY D) 21 November 1995 (1995-11-21)
D10 MUECK A O ET AL: "ANGIOGENETIC AND ANTI-ANGIOGENETIC EFFECTS OF ESTRADIOL AND ITS METABOLITES" JOURNAL OF CLINICAL AND BASIC CARDIOLOGY, 2001, vol 4; pages 153-155, XP008004903
D11 BAYARD F (REPRINT) ET AL: "EFFECTS OF ESTRADIOL, ESTRIOL, ESTETROL AND ESTRONE ON A STRAIN OF HUMAN-BREAST CANCER -CELLS IN CULTURE (MCF -7)" ANNALES D ENDOCRINOLOGIE, (1980) VOL. 41, NO. 5, PP. C26., 1880, XP001121532

D1, (US5340585, see column 12, line 25-44; table 1; claims 1,3,4,13; column 13, line 67; column 15, line 1) and D2, (WO9426207, see page 17, line 5-30, table 1; claims 1,4,13; page 19, last line) disclose compositions comprising: an estrogen (estetrol, the preferred compounds of the present invention, is a preferred estrogen) and GNRH. It is clearly stated in column 12, lines 25-26, that these composition can be used to prevent (reduce the risk) breast and ovarian cancer.
D3, (US5340584, see column 14, line 11-43; table 1; claims 1,4,16; column 16, line 11; column 17, line 30; column 7, line 13) discloses compositions comprising estetrol, GnRH and a progestogen and their use to reduce the risk of ovarian cancer.
D6 discloses some estrogens having a formula covering in general terms also estetrol, and the use of these compounds to treat breast cancer.

Novelty Art.33(2) PCT and inventive step Art.33(3) PCT

Claim 1

Claim 1 has been limited to compositions not comprising GnRH.
Basis for the amendment is found in the description of the application as filed page 15, line 31-34. The amendment concerning the definition of the "precursors" of claim 1 is supported by originally filed claim 5, and the amendment concerning the definition of the diseases is supported by original claim 10.

Since D1-D3 only disclose compositions comprising GnRH, and D6 does not disclose specifically compounds falling in the definition of claim 1, novelty is acknowledged.
From D1-D3 (see for example D1, column 11, line 58 - column 12 line 18) it appears that the presence of GnRH is essential to prevent cancer; for this reason the skilled person would not have provided compositions not comprising GnRH. Inventive step is therefore also acknowledged.

D6 (US4937238, see column 3, lines 18-25 and formula 1) discloses the use of estrogens having a Markush formula covering estetrol for the treatment of breast cancer. No specific compound falling in this definition is however disclosed. D6 (see column 3, lines 5-10) defines as suitable estrogens only those competing and displacing estradiol-17-beta from [the receptors] of mammary cells. This statement implies that some of the compounds defined by the claims of D6 are not active (for example estradiol 17-beta itself). This statement also implies that, since it is known that estetrol is a much weaker estrogen compared to estradiol, the skilled person would have not selected compounds like estretrol from the ones defined in D6.
In addition, the strong chemotherapic effects in rats shown by estetrol in the present application are quite surprising. For these reasons, inventive step over D6 is acknowledged.

NOTE. D4 discloses compounds which do not fall in the definition of the compounds of Claim 1. This document (independently from its publication date) is not considered relevant for the assessment of novelty and inventive step of claim 1 (this applies to claims 2 and 3 as well).

Claim 2, 14, 19, 20

Claim 2,14,19,20 relate to compositions, kits and delivery systems comprising a compound as in claim 1 and an aromatase inhibitor, and to the use of these products in a method to treat estrogen dependent tumours. Basis for the new claims can be found in original claims 1, 12,1516,22,23.
The prior art does not mention the administration of this combination of medicaments.

Claim 2,14,19,20 are therefore novel. D1-D3 do not contain any statement which would prompt a skilled person to associate these two medicaments. Also, from D1-D3 is also clear that GnRH is required for reducing the risk of breast and ovarian cancer. The skilled person would therefore not have replaced GnRH with any other agent.
Furthermore, according to the inventors of the present application the combination of estetrol with aromatase inhibitors suppresses endogenous estrogen production and other side-effects i.e. hypoestrogenism induced by aromatase inhibitors (see page 15, line 28-34). This technical effect of compounds of formula 1 was not mentioned nor could be expected from the prior art. For these reasons inventive step is also acknowledged.
Regarding D6, the same reasoning used when dealing with claim 1 applies here.

Claim 3

Claims 3 has been restricted to relate to the use of compounds of compounds as in claim 1 in a method of treatment of estrogen-sensitive tumours ("prevention" has been deleted from the claim).
D1-D3 are clearly limited to the prevention of breast and ovarian cancer, and are completely silent in regard to cancer treatment; claim 1 is therefore new. Also, since compounds useful to reduce the risk of tumours can not be expected to treat these aggressive disease once they have already developed, and since D1-D3 all indicate that GnRH is essential to obtain the preventive effect, inventive step is also acknowledged.
Regarding D6, the same reasoning used when dealing with claim 1 applies here.

The dependent claims, being more restricted then the independent claim from which they depend from, are also deemed to be new and to involve an inventive step.

Note: D5 discloses the same compounds of the present application and their use for the treatment of hypoestrogenism in subjects subject to breast cancer treatment (see page 10, line 18, and claim 5). This document, published after the date of filing of the present application but having an earlier filing date could become relevant during the proceedings before the regional / national authorities.

Industrial application

The subject matter of claims 1-21 is industrially applicable.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P045194PCT DBO/jdo	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/NL 03/00513	International filing date *(day/month/year)* 11.07.2003	Priority date *(day/month/year)* 12.07.2002
International Patent Classification (IPC) or both national classification and IPC A61K31/565		
Applicant PANTARHEI BIOSCIENCE B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.01.2004	28.10.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Veronese, A Telephone No. +49 89 2399-7824

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL 03/00513

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-27 as originally filed

Claims, Numbers

1-21 received on 15.09.2004 with letter of 15.09.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-21
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents; unless otherwise indicated, reference is made to the relevant passages emphasized in the Search Report.

D1: US-A-5 340 585 (PIKE MALCOLM C ET AL) 23 August 1994
D2: WO 94 26207 A (UNIV SOUTHERN CALIFORNIA) 24 November 1994
D3: US-A-5 340 584 (PIKE MALCOLM C ET AL) 23 August 1994
D4: WO 02 100877 A (SOUTHWEST FOUND BIOMED RES) 19 December 2002
D5: WO 02 094276 A (BUNSCHOTEN EVERT JOHANNES); 28 November 2002
D6: US-A-4 937 238 (LEMON HENRY M) 26 June 1990
D7: LIPPERT CAROLINE ET AL: "The effects of A-ring and D-ring metabolites of estradiol on the proliferation of vascular endothelial cells" LIFE SCIENCES, PERGAMON PRESS, OXFORD, GB, vol. 67(13), 18 August 2000 pg 1653-1658.
D8: NAMBARA T ET AL: "SYNTHESIS OF ESTEROL MONOGLUCURONIDES" STEROIDS, MA, US, vol. 27(1), January 1976, pages 111-122, XP009004815
D9 US 5 468 736 A (HODGEN GARY D) 21 November 1995 (1995-11-21)
D10 MUECK A O ET AL: "ANGIOGENETIC AND ANTI-ANGIOGENETIC EFFECTS OF ESTRADIOL AND ITS METABOLITES" JOURNAL OF CLINICAL AND BASIC CARDIOLOGY, 2001, vol 4; pages 153-155, XP008004903
D11 BAYARD F (REPRINT) ET AL: "EFFECTS OF ESTRADIOL, ESTRIOL, ESTETROL AND ESTRONE ON A STRAIN OF HUMAN-BREAST CANCER -CELLS IN CULTURE (MCF -7)" ANNALES D ENDOCRINOLOGIE, (1980) VOL. 41, NO. 5, PP. C26., 1880, XP001121532

D1, (US5340585, see column 12, line 25-44; table 1; claims 1,3,4,13; column 13, line 67; column 15, line 1) and **D2**, (WO9426207, see page 17, line 5-30, table 1; claims 1,4,13; page 19, last line) disclose compositions comprising: an estrogen (estetrol, the preferred compounds of the present invention, is a preferred estrogen) and GNRH. It is clearly stated in column 12, lines 25-26, that these composition can be used to prevent (reduce the risk) breast and ovarian cancer.
D3, (US5340584, see column 14, line 11-43; table 1; claims 1,4,16; column 16, line 11; column 17, line 30; column 7, line 13) discloses compositions comprising estetrol, GnRH and a progestogen and their use to reduce the risk of ovarian cancer.
D6 discloses some estrogens having a formula covering in general terms also estetrol, and the use of these compounds to treat breast cancer.

Novelty Art.33(2) PCT and inventive step Art.33(3) PCT

Claim 1

Claim 1 has been limited to compositions not comprising GnRH.
Basis for the amendment is found in the description of the application as filed page 15, line 31-34. The amendment concerning the definition of the "precursors" of claim 1 is supported by originally filed claim 5, and the amendment concerning the definition of the diseases is supported by original claim 10.

Since D1-D3 only disclose compositions comprising GnRH, and D6 does not disclose specifically compounds falling in the definition of claim 1, novelty is acknowledged. From D1-D3 (see for example D1, column 11, line 58 - column 12 line 18) it appears that the presence of GnRH is essential to prevent cancer; for this reason the skilled person would not have provided compositions not comprising GnRH. Inventive step is therefore also acknowledged.

D6 (US4937238, see column 3, lines 18-25 and formula 1) discloses the use of estrogens having a Markush formula covering estetrol for the treatment of breast cancer. No specific compound falling in this definition is however disclosed. D6 (see column 3, lines 5-10) defines as suitable estrogens only those competing and displacing estradiol-17-beta from [the receptors] of mammary cells. This statement implies that some of the compounds defined by the claims of D6 are not active (for example estradiol 17-beta itself). This statement also implies that, since it is known that estetrol is a much weaker estrogen compared to estradiol, the skilled person would have not selected compounds like estretrol from the ones defined in D6.
In addition, the strong chemotherapic effects in rats shown by estetrol in the present application are quite surprising. For these reasons, inventive step over D6 is acknowledged.

NOTE. D4 discloses compounds which do not fall in the definition of the compounds of Claim 1. This document (independently from its publication date) is not considered relevant for the assessment of novelty and inventive step of claim 1 (this applies to claims 2 and 3 as well).

Claim 2, 14, 19, 20

Claim 2,14,19,20 relate to compositions, kits and delivery systems comprising a compound as in claim 1 and an aromatase inhibitor, and to the use of these products in a method to treat estrogen dependent tumours. Basis for the new claims can be found in original claims 1, 12,1516,22,23.
The prior art does not mention the administration of this combination of medicaments.

Claim 2,14,19,20 are therefore novel. D1-D3 do not contain any statement which would prompt a skilled person to associate these two medicaments. Also, from D1-D3 is also clear that GnRH is required for reducing the risk of breast and ovarian cancer. The skilled person would therefore not have replaced GnRH with any other agent. Furthermore, according to the inventors of the present application the combination of estetrol with aromatase inhibitors suppresses endogenous estrogen production and other side-effects i.e. hypoestrogenism induced by aromatase inhibitors (see page 15, line 28-34). This technical effect of compounds of formula 1 was not mentioned nor could be expected from the prior art. For these reasons inventive step is also acknowledged.
Regarding D6, the same reasoning used when dealing with claim 1 applies here.

Claim 3

Claims 3 has been restricted to relate to the use of compounds of compounds as in claim 1 in a method of treatment of estrogen-sensitive tumours ("prevention" has been deleted from the claim).
D1-D3 are clearly limited to the prevention of breast and ovarian cancer, and are completely silent in regard to cancer treatment; claim 1 is therefore new. Also, since compounds useful to reduce the risk of tumours can not be expected to treat these aggressive disease once they have already developed, and since D1-D3 all indicate that GnRH is essential to obtain the preventive effect, inventive step is also acknowledged.
Regarding D6, the same reasoning used when dealing with claim 1 applies here.

The dependent claims, being more restricted then the independent claim from which they depend from, are also deemed to be new and to involve an inventive step.

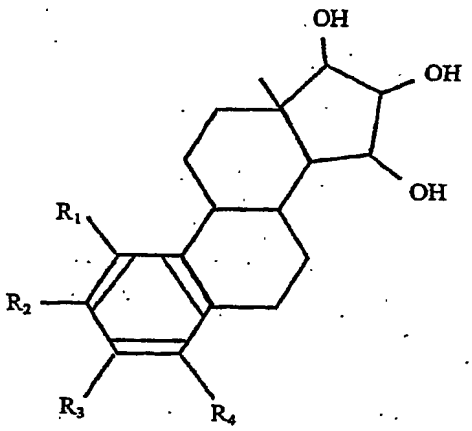
Note: D5 discloses the same compounds of the present application and their use for the treatment of hypoestrogenism in subjects subject to breast cancer treatment (see page 10, line 18, and claim 5). This document, published after the date of filing of the present application but having an earlier filing date could become relevant during the proceedings before the regional / national authorities.

Industrial application

The subject matter of claims 1-21 is industrially applicable.

CLAIMS

1. Use of an estrogenic component selected from the group consisting of:
substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms;

precursors capable of liberating a substance according to the aforementioned formula when used in the present method, which precursors are derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue; and

mixtures of one or more of the aforementioned substances and/or precursors;

in the manufacture of a pharmaceutical composition for use in a method of treating or preventing estrogen-sensitive tumours in a mammal, said estrogen-sensitive tumours being selected from the group consisting of breast cancer and uterine cancer, ovarian cancer, endometriosis, uterine fibroids, benign prostatic hyperplasia and melanoma; and said method comprising the administration of a therapeutically effective amount of the estrogenic component to said mammal and not comprising administration of a GnRH composition.

2. Use of an estrogenic component as defined in claim 1 in the manufacture of a pharmaceutical composition for use in a method of treating or preventing estrogen-sensitive

tumours in a mammal, said estrogen-sensitive tumours being selected from the group consisting of breast cancer and uterine cancer, ovarian cancer, endometriosis, uterine fibroids, benign prostatic hyperplasia and melanoma; and said method comprising the administration to said mammal of a therapeutically effective amount of the estrogenic component in combination with an aromatase inhibitor.

3. Use of an estrogenic component as defined in claim 1 in the manufacture of a pharmaceutical composition for use in a method of treating estrogen-sensitive tumours in a mammal, said estrogen-sensitive tumours being selected from the group consisting of breast cancer and uterine cancer, ovarian cancer, endometriosis, uterine fibroids, benign prostatic hyperplasia and melanoma; and said method comprising the administration of a therapeutically effective amount of the estrogenic component to said mammal.

2. Use according to any one of claims 1-3, wherein no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms;

3. Use according to any one of claims 1-~~4 or 2~~, wherein R_3 represents a hydroxyl group or an alkoxy group.

4.6. Use according to any one of claims 1-~~5~~3, wherein at least 3 of the groups R_1, R_2, R_3 and R_4 represent hydrogen atoms.

~~5. Use according to any one of claims 1-4, wherein the precursors capable of liberating the estrogenic substance are derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue.~~

~~6.~~7. Use according to any one of claims 1-~~6~~5 , wherein the method comprises the uninterrupted administration of the estrogenic component during a period of at least 5 days, preferably of at least 30 days.

7.8. Use according to any one of claims 1-76, wherein the method comprises oral, transdermal, intravenous or subcutaneous administration of the estrogenic component.

8.9. Use according to claim 87, wherein the method comprises oral administration.

9.10. Use according to any one of claims 1-98, wherein the estrogenic component is administered in an amount of at least 1 μg per kg of bodyweight per day, preferably of at least 5 μg per kg of bodyweight per day.

~~10. Use according to any one of claims 1-9, wherein the estrogen-sensitive tumours are selected from the group consisting of breast cancer, uterine cancer, ovarian cancer, endometriosis, uterine fibroids, benign prostatic hyperplasia and melanoma.~~

11. Use according to any one of claims 1-10,9 wherein the estrogen-sensitive tumours are selected from the group consisting of breast cancer and uterine cancer.

~~12. Use according to any one of claims 1-11, wherein the method comprises co-administration of an estrogen suppressant in an amount effective to suppress the endogenous estrogen production, wherein said estrogen suppressant is selected from the group consisting of progestogens, GnRH analogues, aromatase inhibitors, cyclo-oxygenase 2 (COX-2) inhibitors, 17β-hydroxysteroid dehydrogenase type 1 inhibitors and combinations thereof.~~

~~13.~~12. Use according to claim 2~~11~~, wherein the aromatase inhibitor~~estrogen suppressant~~ is co-administered in an effective amount to suppress blood serum 17β-estradiol level to below 10 pg/ml, more preferably to below 5 pg/ml, most preferably to below 1 pg/ml

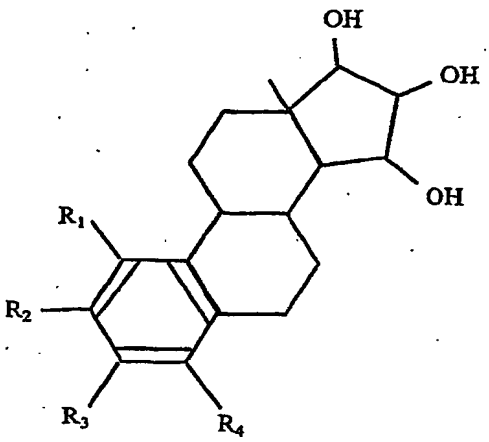
~~14. Use according to claim 11 or 12, wherein the method comprises co-administration of a progestogen~~

~~15.~~13. Use according to claim 1 or 3~~1 or 12~~, wherein the method comprises co-administration of an aromatase inhibitor.

~~16.~~14. A pharmaceutical composition containing:

a. at least 0.01 mg of an ~~estrogen suppressant selected from the group consisting of~~ aromatase inhibitors~~, GnRH analogues and combinations thereof~~;

b. at least 0.05 mg of an estrogenic component selected from the group consisting of: substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms;

precursors capable of liberating a substance according to the aforementioned formula when used in the present method, which precursors are derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue; and mixtures of one or more of the aforementioned substances and/or precursors; and

c. pharmaceutically acceptable excipient.

~~17.~~15. The pharmaceutical composition according to claim 1~~4~~6, wherein no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms.

~~18.~~16. The pharmaceutical composition according to claim 1~~4~~6 or 1~~5~~7, wherein R_3 represents a hydroxyl group or an alkoxy group.

~~19.~~17. The pharmaceutical composition according to any one of claims 1~~4~~6-1~~6~~8, wherein at least 3 of the groups R_1, R_2, R_3 and R_4 represent hydrogen atoms.

~~18. The pharmaceutical composition according to any one of claims 16-19, wherein the precursors capable of liberating the estrogenic substance are derivatives of the present estrogen substances, wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranyl; or a straight or branched chain glycosydic residue containing 1-20 glycosidic units per residue.~~

~~21.~~18. The pharmaceutical composition according to any one of claims 1~~4~~6-17~~20~~, wherein the composition contains aromatase inhibitor in an amount equivalent to an oral dosage of at least 0.05 mg anastrozole.

~~22.~~19. A drug delivery system comprising a pharmaceutical composition according to any one of claims 1~~3~~6-~~2~~17, said drug delivery system being selected from the group consisting of an oral dosage unit; an injectable fluid; a suppository; a pessary; a gel; and a cream.

~~23.~~20. A pharmaceutical kit comprising one or more dosage units containing at least 0.05 mg of the estrogenic component as defined in claim 1 and a pharmaceutically acceptable excipient; and one or more dosage units containing at least 0.01 mg of an ~~estrogen suppressant selected from the group consisting of GnRH analogues,~~ aromatase inhibitors~~, cyclo-oxygenase 2 (COX-2) inhibitors, 17β-hydroxysteroid dehydrogenase type 1 inhibitors and combinations thereof~~, and a pharmaceutically acceptable excipient.

~~24.~~21. The pharmaceutical kit according to claim 20~~23~~, wherein the dosage units are oral dosage units.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 03/00513

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/565 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 340 585 A (PIKE MALCOLM C ET AL) 23 August 1994 (1994-08-23) column 12, line 25-44; table 1 claims 1,3,4,13 column 13, line 67 column 15, line 1 ---	1-4, 6-10, 12-19, 22-24
X	WO 94 26207 A (UNIV SOUTHERN CALIFORNIA) 24 November 1994 (1994-11-24) page 17, line 5-30; table 1 claims 1,4,13 page 19, last line --- -/--	1-4, 6-10, 12-19, 22-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2003	07/10/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Veronese, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 340 584 A (PIKE MALCOLM C ET AL) 23 August 1994 (1994-08-23) column 14, line 11-43; claims 1,4,16 table 1 column 16, line 11 column 17, line 30 column 7, line 13	1-4, 6-10, 12-19, 22-24
P,X	WO 02 100877 A (SOUTHWEST FOUND BIOMED RES) 19 December 2002 (2002-12-19) page 15, line 15 -page 16-30; claims 28,73,80,81	1,3,5-10
P,X	WO 02 094276 A (BUNSCHOTEN EVERT JOHANNES ;COELINGH BENNINK HERMAN JAN TI (NL); PA) 28 November 2002 (2002-11-28) *See page 10, lines 18-19: breast cancer* claims 1-15 See claim 5, cancer *	1-11
X	US 4 937 238 A (LEMON HENRY M) 26 June 1990 (1990-06-26) column 1, line 63 -column 2 column 3, line 19-25	1-4,6-11
X	LIPPERT CAROLINE ET AL: "The effects of A-ring and D-ring metabolites of estradiol on the proliferation of vascular endothelial cells" LIFE SCIENCES, PERGAMON PRESS, OXFORD, GB, vol. 67, no. 13, 18 August 2000 (2000-08-18), pages 1653-1658, XP002200988 ISSN: 0024-3205 See table 1, estetrol abstract page 1657, last paragraph	1-10
A	NAMBARA T ET AL: "SYNTHESIS OF ESTEROL MONOGLUCURONIDES" STEROIDS, BUTTERWORTH-HEINEMANN, STONEHAM, MA, US, vol. 27, no. 1, January 1976 (1976-01), pages 111-122, XP009004815 ISSN: 0039-128X * See charts 1-3 *	5
A	US 5 468 736 A (HODGEN GARY D) 21 November 1995 (1995-11-21) column 3, line 5-10 example 3 claims	12-19

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MUECK A O ET AL: "ANGIOGENETIC AND ANTI-ANGIOGENETIC EFFECTS OF ESTRADIOL AND ITS METABOLITES" JOURNAL OF CLINICAL AND BASIC CARDIOLOGY, XX, AU, vol. 4, 2001, pages 153-155, XP008004903 ISSN: 1561-2775 * See table 1, estetrol * page 154, column 1	1
A	BAYARD F (REPRINT) ET AL: "EFFECTS OF ESTRADIOL, ESTRIOL, ESTETROL AND ESTRONE ON A STRAIN OF HUMAN-BREAST CANCER -CELLS IN CULTURE (MCF -7)" ANNALES D ENDOCRINOLOGIE, (1980) VOL. 41, NO. 5, PP. C26., 1880, XP001121532 CTR HOSP UNIV TOULOUSE RANGUEIL, INSERM, U168, F-31054 TOULOUSE, FRANCE the whole document	1

INTERNATIONAL SEARCH REPORT

ational application No.
PCT/NL 03/00513

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: –
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The expression "precursors" in claims 1 relates to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds defined by this expression. The expression estrogen - sensitive tumors does not clearly define the tumors for which protection is intended (benign prostatic hyperplasia and endometriosis in claim 10 are even not tumors). Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds having the formula given in claim 1, the ones definded in claim 5, and their use in relation to the treatment of the diseases mentioned in claim 10-11.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 03/00513

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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